THE GERMICIDAL ACTIVITY OF THE EUCALYPTUS OILS.

Part ii. The Action of the Oils in Aqueous Dilutions.

By R. Greig-Smith, D.Sc., Macleay Bacteriologist to the Society.

(With seven Text-figures).

In Part i. of this contribution, I dealt with the germicidal activity of the Eucalyptus oils when dissolved in a neutral oil such as olive oil, and it was shown that under such conditions they were poor disinfectants. The phenol-coefficients, against *B. coli communis* at 20° for two hours, ranged from 0·4 with the oils of *E. linearis*, *E. cinerea*, rect., and *E. australiana*, crude, 3rd hour, down to 0·07 with cineol and the oil of *E. polybractea*.

In this paper, an examination has been made of their germicidal powers when in aqueous dilution.

It is a difficult matter to determine the real, hygienic or economic value of a disinfectant, for so much depends upon the material in which the bacteria are contained. They may be suspended in blood, pus, sputum, urine, sewage, water, trade waste, etc., all of which have variable influences in absorbing or rendering inert the disinfectant. The proteids and fat are among the most active destroyers of the disinfectants, and in considering their virtues the nature of the bacterial menstruum or, as it is called, their environment, has to be taken into account. The coal tar products, for example, have their powers seriously reduced by fat, in which they are more soluble than in water, while the metallic disinfectants, such as mercuric chloride, are weakened by proteids with which they form compounds. The oxidising disinfectants are more or less used up in oxidising the organic

matter of the environment. It is, therefore, evident that a common medium must be employed when a comparison is desired, and the Rideal-Walker test is that which is generally used. In it, the activity of the disinfectant is not interfered with, and although the test has its weaknesses, it has been very useful in giving us some idea of the value of disinfectants in the absence of organic matter. The test is easy to do, and it should give approximately similar results when made by different workers.

The Eucalyptus oils are partly soluble in water and partly emulsified, much depending upon the oil and upon the quantity of water. Cineol or Eucalyptol, the chief constituent of many of the oils, is soluble in about 300 parts of water, while the other constituents are so insoluble that many give opalescent dilutions with 2,000 parts of water. Their power of forming emulsions is probably of value, for, as I think Martin has pointed out, the adsorption by bacteria from emulsions is greater* than from solutions, and the adsorption is the first step in the destruction of bacteria by disinfectants.

In preparing the dilutions of the oils, two methods were employed—the mass and the droplet methods. In the first, a certain weight of oil was shaken with a calculated quantity of water, usually 199 times the weight of the oil, and from this strong emulsion the weaker dilutions were made by adding the requisite quantities of water. In working with the oils, one is struck with the tenacity with which they adhere to the glass of the flasks and pipettes, and with the idea of minimising any irregularity rising from this phenomenon, the droplet method was used as an alternative. One point in favour of the droplet method is that, when only small quantities of the oil are available, there is very little waste. A capillary pipette was made and kept for dropping. Each oil under examination was tested daily or weekly, according to the laboratory temperature, by

* "Adsorption undoubtedly plays a large part in many forms of disinfection, and confers upon emulsions, as contrasted with solutions, considerable advantages." Somerville, Cantor Leetures, 1913. Roy. Soc. of Arts.

weighing 30 drops discharged from the pipette while held vertically. Three weighings were made, and as these never varied from a milligram or two, the weight of an average droplet was obtained. The volume of water necessary to make about 10 c.c. of the required dilution was pipetted into a wide-mouthed ounce bottle, and the requisite number of drops of oil were added. The bottle was corked and shaken 300 times at intervals and finally before the abstraction of 2 c.c. This quantity was pipetted into a small tube, $5 \times \frac{5}{8}$ inches, a drop* of a 20 hours' broth culture of *B. coli communis* was added, and the tube was put into a water-bath at 20° for the allotted time.

At certain intervals the dilutions were shaken and a small quantity withdrawn from each by means of a platinum spiral,† and put into a test tube containing 3 c.c. of Lemco broth. The infected Lemco tubes were incubated at 37° for two days.

The dilutions were generally made in steps of 100 up to 1,000, then by 200 up to 2,000, but in certain cases this was departed from and the steps made smaller, as in the case of cineol and phenol.

When the oils are diluted with water, they are more or less opalescent, depending upon the relative amounts of oil and water. The oil slowly dissolves, and the faintly opalescent dilutions become clear.

The question arose as to when the dilutions should be tested, that is to say, at what time, after their preparation, were they most potent? To answer this question, cineol was tested, and it was found to be as effective in twenty minutes as in four hours, but that it became less and less germicidal as the time extended from one to twelve days. This is in keeping with the idea that an emulsion of a disinfectant is more effective than a solution, and, with cineol, the potency seemed to keep pace with the solubility. Those dilutions which just became bright at 12°

* From a capillary pipette discharging 40 drops of broth per gram.

† Made by rolling an ordinary platinum needle wire five times round a No. 18 wire. It withdrew 7 milligrams of broth. Several spirals were used, so that one was always cold. They were consistent in picking up the same weight of liquid.

were just germicidal to *B. coli communis* at 20° in 15 minutes. It was decided that the dilutions should be tested within an hour after their preparation.

Some further preliminary work was done to determine which were the best strengths of some oils to employ, and in how far we might expect to get consistent results. The lethal dilutions vary more or less in each experiment, and it is sometimes difficult to determine what is the real efficiency of the oil under examination. At one time, it was considered that the lowest of all the dilutions germicidal with a certain exposure would indicate the efficiency, but the curve of these dilutions was often too irregular,* and did not bear out the idea given by the curves of the individual tests. Consequently the germicidal dilutions of each test were plotted, and the probable dilution curve drawn through at least three exposures. From this curve the dilutions were corrected for the other exposures. The average lethal dilution for each exposure was calculated from the corrected lethal dilutions, and the curve of these numbers was taken as passing through the probable effective dilution.

Cineol and Phenol.—The cineol was obtained from Mr. G. I. Hudson, and had been obtained by freezing the rectified oils of *E. polybractea* or of *E. sideroxylon*. The phenol was obtained as crystals of phenol absolute which in the preliminary experiment solidified at 38·5°,† and in the others at 40°.

* Lee and Gilbert (Abs. Journ. Soc Chem. Ind., 1918, 439A) find that disinfection is an orderly time process analogous to a chemical reaction. A definite relationship exists between the velocity of the reaction and the concentration of the disinfectant. Chick (Journ. Hyg., 1910, 237) found the same analogy and found that the velocity of disinfection at any moment was proportional to the number of surviving bacteria. Disinfection proceeded in accordance with a logarithmic law of the first order, *i.e.*, like a chemical reaction.

† Noted in Part i. as being crystalline at 28°.

TABLE i. Preliminary Experiment. *B. coli communis.*

Exposure in minutes.		15	30	60	120	180	240
Cineol, *a*.	1 :	260	—	300	—	380	—
b.	1 :	260	—	340	360	420	420
c.	1 :	240	—	320	340	360	380
d.	1 :	260	280	300	—	—	—
Probable effective dilution	1 :	255	280	315	355	385	410
Phenol, *a*. ...	1 :	90	100	120	120	120	140
b. .. .	1 :	70	80	90	110	120	140
c.	1 :	—	90	110	120	140	140
Probable effective dilution	1 :	80	92	107	123	132	137
Phenol coefficient of cineol	1 :	3·2	3·1	3·0	2·9	2·9	2·9

These preliminary tests were made in October; in the following March, the activity was again determined, with the new lot of phenol.

TABLE ii. *B. coli communis.*

Exposure in minutes.		15	30	45	60	120	180	240
Cineol, 19/3 ...	1 :	300	350	—	425	425	425	425
20/3 ...	1 :	275	275	—	375	450	450	450
21/3	1 :	275	275	300	375	375	375	375
26/3 ...	1 :	275	375	400	425	450	450	450
27/3 ...	1 :	250	325	350	350	350	400	425
Probable effective dilution	1 :	275	350	375	392	415	420	425
Phenol. 14/3 ...	1 :	100	100	—	120	130	150	—
18/3 ...	1 :	100	100	—	110	130	130	140
19/3 ..	1 :	90	110	—	120	130	150	160
20/3 ...	1 :	90	110	—	120	130	140	160
28/3 ...	1 :	90	100	120	120	140	140	150
Probable effective dilution	1 :	90	104	111	118	135	145	152
Cineol. Second lot, freshly prepared 25/3	1 :	225	325	350	350	400	—	—
26/3	1 :	250	300	325	375	375	425	425
27/3	1 :	225	275	375	375	400	400	400
28/3	1 :	275	275	325	325	350	350	350
1/4	1 :	275	325	350	400	400	450	450
Probable effective dilution	1 :	245	325	355	375	400	405	410
Phenol coefficient of cineol		3·1	3·4	3·4	3·3	3·1	2·9	2·8
cineol. Second lot		2·7	3·1	3·2	3·2	3·0	2·8	2·7

Stronger dilutions were tested at shorter intervals, but as the tube method, on account of the apparent necessity for keeping the oil emulsified, was considered impracticable, wide-mouthed ounce-bottles containing 10 c.c. of emulsion were cooled to 20°, and each was treated with five drops of a 20-hour broth culture and shaken repeatedly during the period of exposure. The results were very irregular, much more so than with more dilute emulsions, and the reason for this was not at once elucidated. One test showed that a greater amount of shaking lessened the lethal exposures, but when the shaking was regulated, the results were no more uniform. Differences in the reaction of the broth, which is claimed by J. H. Wright* to cause discrepancies in the results obtained in testing disinfectants, did not account for those obtained by me, for tests were made with dilutions of cineol, 1 : 100, which after exposures of 2, 4, 6, 8, and 10 minutes, were seeded into tubes of broth varying in reaction from +3° through 6°, 8, 10° to +12°, and all showed no growth in 6 minutes.

The phenol tests were always fairly constant; in fact the broth cultures used for infecting the tests were controlled by the bacteria being destroyed in 6 minutes by a 1 : 80 solution of phenol at 20°. If a shorter or a longer time was taken to destroy the bacteria, the experiment was rejected, and the cultures examined to obtain a mixture of races capable of being destroyed under the conditions in six minutes.

In considering the uniformity of the phenol tests and the comparative regularity of the tests with the weaker dilutions of cineol, it would appear that an irregularity of result is to be expected in dealing with strong emulsions which separate so readily as do mixtures of cineol and distilled water. I have, therefore, recorded the results of the tests, irregular as they are, and have calculated the general average which enabled a curve of probable efficiency to be made.

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* Through Abs. of Bact., 1918, 78.

TABLE iii. *B. coli communis.*—Cineol with short Exposures.

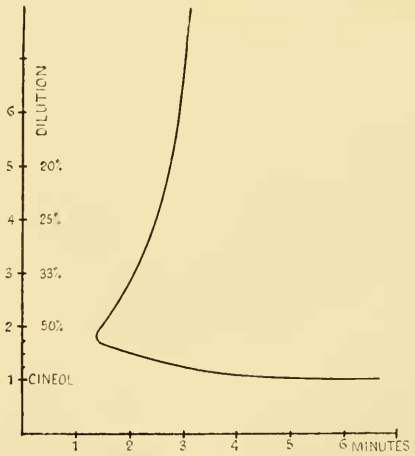
Dilutions... ... ... 1 :	1	1¼	1½	2	5	10	25	30	50	75	100	150	200	225	250	275	300
Cineol, 1st lot	10	—	—	½	2½	2½	4½	—	4½	—	5	6	5	—	—	—	—
	—	—	—	½	2	2½	—	—	4	—	—	—	—	—	—	—	—
	—	—	—	—	1½	3½	6	—	6	—	—	—	—	—	—	—	—
	—	—	—	1½	4	—	5	—	4½	5	4	—	—	—	—	—	—
	—	—	—	1½	2½	—	3	—	4½	—	—	5	5	—	—	—	—
	—	—	—	1½	3½	—	3	—	3	—	2½	4½	7	—	—	—	—
	—	—	—	—	3	—	—	—	3	—	6	—	—	—	—	—	—
	—	—	—	2	4½	—	5	—	4	3½	—	—	—	—	—	—	—
	—	—	—	1½	2½	—	3	—	4	—	3½	—					
2nd lot	—	—	—	2	3	5	—	4	8	—	8	10	8	10	10	10	—
	—	—	—	2	4	4	—	5	6	—	5	7	5	—	9	—	—
	—	—	—	2	3	—	—	6	9	—	6	6	4	6	15	15	15
	—	—	—	2	3	2	—	—	4	—	4	6	4	6	6	10	15
	30	3	2	2	—	—	—	—	4	—	4	4	4	—	10	15	—
	—	—	—	2	3	3	—	4	3	—	5	—	10	—	—	—	—
	—	—	—	1	3	2	—	2	2	—	5	—	8	—	—	—	—
	—	—	—	—	—	—	—	—	4	4	4	5	6	—	—	12	—
	—	—	—	—	—	—	—	—	3	4	6	5	8	—	—	10	—
	—	—	—	—	—	—	—	—	6	5	4	6	6	—	—	12	—
	—	—	—	—	—	—	—	—	6	6	5	6	10	—	10	20	—
	5	2	—	2	3	4	—	3	5	—	5	4	10	—	10	—	—
	5	3	2	2	3	3	—	3	4	—	—	—	—	—	—	—	—
General average	—	3	2	1½	3	3	4	4	4½	4½	5	6	6½	7	10	13	15
Probable effective exposures ...	5+	3	2	1½	2¾	3¼	4	4	4½	4¾	5	5¾	7	8	10	12½	17

TABLE iii.*a*. *B. coli communis*.
Phenol with short Exposures.

Dilutions 1 :	60	70	80	90
	½	1½	5	—
	—	1	6*	10
	½	1½	6	15
Probable effective exposure ...	½	1½	6	15

* Many times.

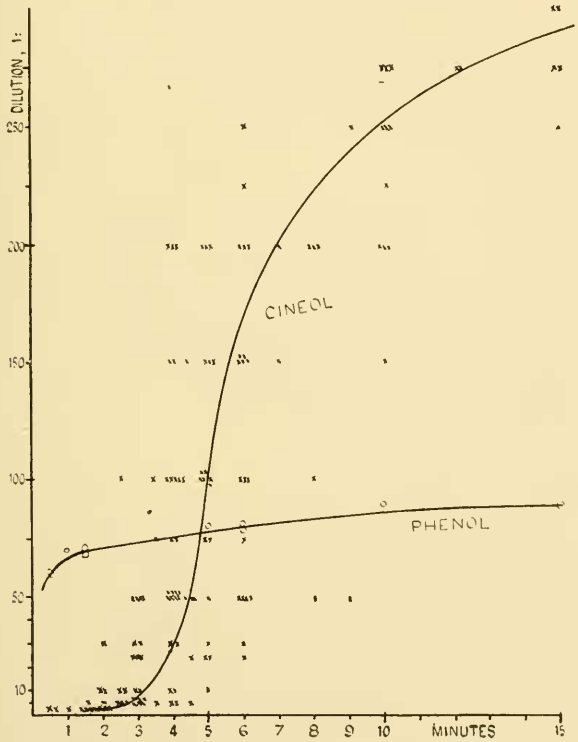
As determined from the results on Table iii., the probable effective exposures for cineol at 15 and 30 minutes are slightly higher than those as determined from Table ii., but this difference is the result of the methods, the bottle giving a speedier distribution than the tube, and consequently a higher efficiency.



Text-fig. i.—Cineol with Small Quantities of Water.

The necessity for the presence of a sufficiency of water in accelerating the action of cineol is shown by the fact, as determined by Cuthbert Hall, that when comparatively dry bacteria, such as are taken from an agar culture, are added to pure cineol, the lethal exposure may vary up to eight hours. With the small quantity of water contained in a few drops of a broth culture,

the disinfecting time varied from 5 to 30 minutes. An 80% emulsion, that is, four parts by weight of cineol to one of water,* had a lethal period of 3 minutes, a 66% emulsion had 2 minutes, and a 50% had 1½ minutes. Then the period began to lengthen as the cineol percentage became smaller. The expressions 1 : 2, 1 : 5, and so on, which occur in the tabulated results, mean 1 in 2 and 1 in 5 by weight of oil and water.

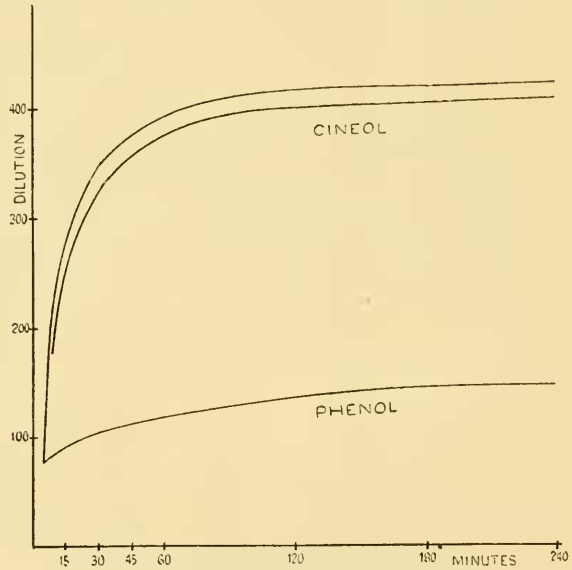


Text-fig. ii.—Cineol with Short Exposures.

The curve of cineol rises slowly from the turning point at one and a half minutes to the three minute interval before rising very rapidly. This lag or period of quiescence is met with in many disinfectants. The rapidity of the rise from the three

* Tabulated as 1 : 1¼.

minute to the eight minute interval explains much of the great irregularity in the tests. Concordant results can scarcely be expected during an interval of rapidly altering activity. The same gross irregularity was not noted with the weaker dilutions of Table ii., or with phenol which furnishes a true solution in dilutions over 1 : 5.



Text-fig. iii.—The Cineol and Phenol Curves.

By combining the results of Tables ii. and iii., we are able to obtain the complete curves of cineol and phenol. Both are of the same nature, inasmuch as they show a sharp rise followed by a slowing down to a straight line rising slightly from the horizontal. Indeed, if we were to enlarge the phenol area, we would find that it had much the same shape as the cineol area. The phenol curve reaches the approximately horizontal level in $1\frac{1}{2}$ minutes, while cineol takes half-an-hour. Although more powerful as a disinfectant than phenol, it is slower in its action and overtakes the quickly acting phenol at the five-minute interval when each has a dilution of 1 : 75.

It is customary, when comparing the germicidal properties of some disinfectants, to refer to them in terms of phenol, which for many reasons is taken as a standard. The phenol-coefficient is obtained by dividing the weakest dilution of a disinfectant which destroys a bacterium in a certain time and at a certain temperature by the weakest dilution of phenol lethal in the same time and at the same temperature. In Table ii. the coefficients have been obtained by dividing the probable effective dilutions of cineol by those of phenol.

The coefficiency curve is comparatively high and reaches a maximum of 3·4 in thirty minutes, and slowly falls to 2·8 in four hours. As Delepine* reminded us, the ratio between the lethal doses of two disinfectants is not the same for exposures of different durations.

There is no definite rule as to the exposure which should be taken in calculating the phenol coefficient of any disinfectant. The method suggested by Rideal and Walker is to withdraw portions of the tests every two and a half minutes up to fifteen, and to take the dilutions of the disinfectants which are lethal at any one exposure. Thus the coefficient might be calculated from any $2\frac{1}{2}$ minute period up to 15 minutes. Sims Woodhead† determined the coefficients at $2\frac{1}{2}$ and at 30 minutes and took the average. Blyth‡ used the $12\frac{1}{2}$ minute interval. Delepine§ preferred exposures of not less than 20 minutes for bacteria such as *B. coli communis*, and took 20 or 30 minutes as the proper exposure, but he recognised that half-hourly and hourly exposures had the advantage of giving more steady results. Chick and Martin‖ considered that 30 minutes was best. A study of the coefficients of cineol leads one to the same conclusion. It would clearly be unwise to take a time during the rapid rise in the cineol curve. It should rather be taken when the curve has begun to assume its nearly horizontal position. The curves for

* Journ. Soc. Chem. Ind., 1910, 1344.

† Lancet, 1909, 1454.

‡ Journ. Soc. Chem. Ind., 1906, 1183.

§ *Idem*, 1911, 334.

‖ Journ. Hyg., 1908, 654.

aromadendral and piperitone are like cineol in this respect, but the Eucalyptus oils generally are different, their curves usually showing a more sustained rise. While theoretically the real coefficient should be the highest, practically we want to know the maximum efficiency in the shortest time so that there might be a minimum of risk in the disinfection of infectious material. The half-hour interval is clearly indicated for cineol and, as we shall see later, for aromadendral and piperitone, as well as for the pinene and sesquiterpene oils.

The question of the choice of cineol as a disinfectant will depend upon circumstances. While phenol forms clear solutions with water in dilutions of 1 : 20 and weaker, cineol, according to the data of Earle,* would at 20° give a clear solution with 1 : 270. The strongest clear solution of cineol destroys *B. coli communis* in 15 minutes at 20°, while the strongest clear solution of phenol kills it instantly. On the other hand, a 1 : 270 dilution of phenol would not destroy the bacillus within a reasonable time. In dilutions stronger than 1 : 270, cineol forms milky emulsions which throw oil globules to the surface upon standing for a few minutes, and on this account it does not give what is termed an elegant preparation.

The oil of E. cinerea.—The crude oil of *E. cinerea* usually contains over 50% of cineol, the remainder being pinene, with a comparatively large amount of esters and some sesquiterpene. The first specimen to be examined was that which gave the high toxicity numbers when tested in oil. It had an acidity of 95°, and, judging by this, some time, probably some years, had passed since its preparation. Two and a half months after the first set of tests, the oil was treated with dry sodium carbonate for two days and dilutions were made by two methods, the mass and the droplet. The idea was to test the two methods, and at the same time to slightly vary the conditions of the experiment. So far as the method is concerned, it appears to be immaterial which is used, as *a*, *b*, *c*, and *e* were made by the droplet and *d* by preparing a bulk dilution of 1 : 200 and making the other dilutions from that.

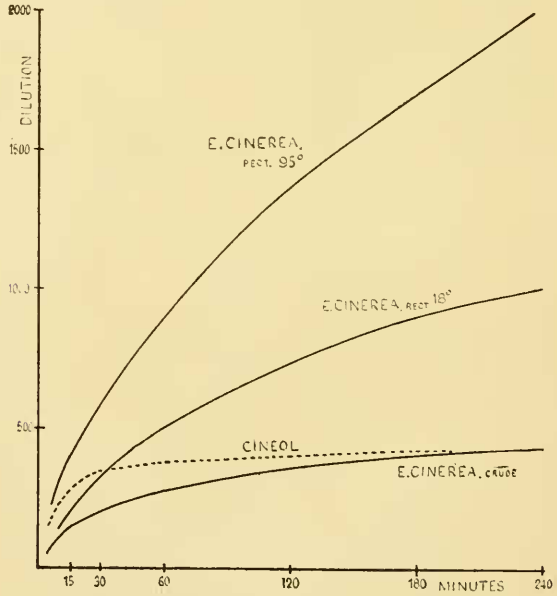
* Journ. Soc. Chem. Ind., 1918, 274T.

TABLE IV. *B. coli communis.*

		Dilutions bactericidal at 20°.										
Exposure in minutes		15	30	45	60	90	120	150	180	210	240	300
E. cinerea, rect., acidity = 95°	*a.* 1:	500	600	700	900	1200	*1200†	—	*1200†	—	—	—
	b. 1:	400	800	900	900	1000	1000	1400	1400	2000*	2000*	—
	c. 1:	400	500	700	1000	1000	1400	1600	1600	2400	2400	—
Probable effective dilution	1:	400	600	800	900	1150	1350	1500	1700	1850	2000	—
The same treated with Na_2CO_3	*d.* 1:	300	600	—	1000	—	1400	—	1600	—	2400	—
	e. 1:	400	500	—	1000	—	1400	—	1600	—	1800	—
E. cinerea, rect., acidity = 18·5°	*f.* 1:	200	—	—	400	—	—	—	800	—	—	1000
	g. 1:	200	—	—	500	—	800	—	1000	—	—	1400
	h. 1:	200	—	—	500	—	600	—	700	—	1200	1200
	k. 1:	200	—	—	700	—	700	—	900	—	1100	1100
Probable effective dilution	1:	200	330	—	500	—	725	—	900	—	1000	1100

* = or weaker, † = but stronger than 1 : 2000.

The older oil is undoubtedly the stronger germicide, and this is not on account of the great amount of acid which it contained, because the neutralisation of the acid did not appreciably alter the efficiency. The crude oil of *E. cinerea* has, compared with many other oils, a high saponification number, 14·4 to 24,* which indicates a preponderance of esters. These apparently become hydrolysed in course of time, producing acids and alcohols, and it is either to the alcohols or to their oxidation products that the increased germicidal effect is to be traced, for, as we shall see later, the aldehydes are the most bactericidal components of the oils.



Text-fig. iv.

It had been suggested that as the crude oils in most cases contain substances of a phenolic nature, they would probably be more germicidal than the rectified oils. When the crude oil of

* Baker and Smith. The Eucalypts and their Essential Oils.

E. cinerea was tested, the results did not bear out the contention. Two specimens were tested.

TABLE V. *B. coli communis.*

			Dilutions bactericidal at 20°.						
Exposure in minutes ...		...	15	30	60	120	180	240	300
E. cinerea, crude, acidity = 81	*a.*	1 :	150	—	250	400	400	400	400
	b.	1 :	150	175	250	350	350	350	350
Probable effective dilution		1 :	150	175	250	350	390	400	400
E. cinerea, crude, acidity = 12°	*a.*	1 :	150	200	300	400	500	—	—
	b.	1 :	150	—	200	350	350	400	500
	c.	1 :	175	—	350	350	350	450	450
Probable effective dilution		1 :	150	200	275	350	400	425	450

Compared with the rectified oils, they have a low germicidal value. Furthermore, while the rectified oil with the higher acid content was the better, the crude oil with 81° of acidity was rather less germicidal than that with 12°. The acidity cannot therefore be depended upon as an index of the disinfecting value of the oil. It would almost seem as if the act of distilling the oil with steam had brought about the production of germicidal substances from the components of the oil. So far, these specimens of oil, both rectified and crude, had not been directly related one with the other, and, therefore, one could only guess at what might be the reason for their different behaviours. But Mr. H. G. Smith had two specimens, one a rectified oil, another the crude oil from which it was obtained, and it was thought that an examination of these might throw some light upon the matter.

TABLE vi. *B. coli communis.*

			Dilutions bactericidal at 20°.					
Exposure in minutes			15	30	60	120	180	240
E. cinerea, crude, acidity = 86°	*a.*	1 :	300	300	400	400	400	600
	b.	1 :	200	200	300	300	300	400
	c.	1 :	200	300	400	500	500	500
Probable effective dilution		1 :	230	300	370	440	470	500
E. cinerea, rect., acidity = 26°	*a.*	1 :	100	300	400	400	500	900
	b.	1 :	100	200	300	400	400	700
Probable effective dilution		1 :	150	250	340	430	450	—

The relative activities of the two oils were very much the same. One must believe that distillation had no influence upon the hitherto observed higher efficiency of the steam-rectified oils.

Superheated steam has likewise no action, as was shown by an experiment in which the crude oil of *E. cinerea* (acidity = 12°) was, with the addition of a few drops of water, sealed in glass tubes and heated at 180° for an hour. Control tubes were sealed at the same time. Six weeks afterwards, the tubes were opened and the oils centrifugalised and examined. The heated oil was found to be slightly more germicidal, but not sufficiently so to warrant the idea that any tangible increase had actually been obtained.

It may be remembered that, when testing the activity of the oils in solution of olive oil, *E. cinerea* was found, after the addition of acetic acid, to become more and more germicidal in course of time. Small quantities of the treated oils remained, and that to which the least quantity of acetic acid had been added, was tested in aqueous dilution.

TABLE vii. *B. coli communis.*

	Dilutions bactericidal at 20°.					
Exposure in minutes	15	30	60	120	180	240
E. cinerea, crude. Acidity 12°, increased to 93° 1 :	200	400	600	800	800	1000
Control as previously determined 1 :	150	200	300	350	400	400

It is clear that a considerable elevation of the germicidal activity had, in course of time, resulted from the acidification. It was tested on the 108th day after acidification. The acidity of the oil would have little effect in acidifying the dilutions, for at 1 : 400 the acidity would be less than 0·25°. The experiment therefore shows that acidification does, in time, cause the oil of *E. cinerea* to become more germicidal, and since storage of the oil results in the natural development of acidity, it follows that storage of the oil, by reason of the formation of acids, will lead to the production of germicidal substances. It will in progress of time become more and more bactericidal.

The Oils of E. australiana.—The oils of this member of the Peppermint group of Eucalypts differ according to whether the trees grow on the high ridges of the Main Dividing Range or at lower elevations. The oil from the latter is termed *E. australiana*, and that from the ridges is named *E. australiana*, Braidwood. *E. australiana* contains some 70% of cineol, while the Braidwood oil has about half this amount replaced by phellandrene. Otherwise the constituents are much the same. Besides the cineol, the oil of *E. australiana* contains a little piperitone, occasionally a little phellandrene, a phenol (Tasmanol), a small amount of esters, and some of an unknown alcohol. The oil is not generally rectified, but, in distilling the leaves, the oil that comes over during the first hour is sufficiently pure to enable it to be sold for medicinal purposes. It is known as "First Hour Oil," and contains over 70% of cineol and usually but a trace of phellandrene and a minimum amount of volatile aldehydes.*

* Proc. Roy. Soc. N. S. Wales, 1915, 514.

TABLE viii. *B. coli communis.*

		Dilutions bactericidal at 20°.						
Exposure in minutes ...	...	15	30	60	120	180	240	300
E. australiana, 1st hour oil, acidity = 5°	*a.* 1 :	400	500	500	600	600	600	600
	b. 1 :	200	400	600	700	700	700	700
	c. 1 :	200	200	400	400	500	500	—
	d. 1 :	200	300	400	500	500	500	—
	e. 1 :	300	300	300	400	500	500	—
Probable effective dilution	1 :	225	350	450	525	550	550	—

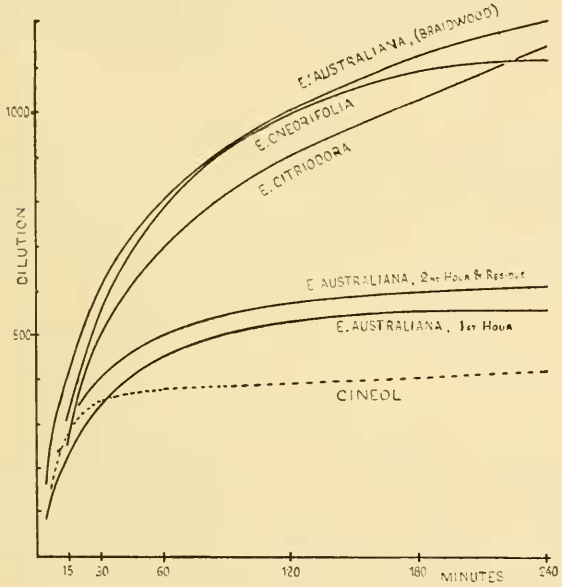
A second specimen of the First Hour Oil was obtained, and it was tested with the second hour oil and the commercial residue.

TABLE ix. *B. coli communis.*

		Dilutions bactericidal at 20°.						
Exposure in minutes	...	15	30	60	120	180	240	
E. australiana, 1st hour, acidity = 10°	*a.* 1 :	200	200	200	400	400	400	
	b. 1 :	300	300	400	500	600	700	
	c. 1 :	300	300	400	500	500	500	
	d. 1 :	300	400	500	500	700	700	(race 7)
Probable effective dilution	1 :	250	325	400	480	520	550	
E. australiana, crude, 2nd hour, acidity = 150°	*a.* 1 :	200	300	300	400	400	500	
	b. 1 :	400	400	400	400	600	600	
	c. 1 :	300	—	500	550	600	600	
	d. 1 :	400	400	500	600	600	800	
Probable effective dilution	1 :	300	400	500	570	590	600	
E. australiana, commercial residue	1 :	300	400	500	600	600	600	

From a germicidal point of view, these oils of *E. australiana* are all the same, and it would appear that the bactericidal constituents are distilled in constant proportions.

The high acidity of the 2nd hour oils will cause the stronger dilutions to be pronouncedly acid; a dilution of 1 : 300 will have an acidity of 0·5° which, by increasing the lethal power, might raise the germicidal dilution considerably (see p.339).



Text-fig.5.

The oil of the Braidwood variety of *E. australiana* was tested to complete the information respecting the oil, and incidentally to see how the replacement of the cineol by phellandrene would affect the germicidal value of the oil.

TABLE X. *B. coli communis.*

		Dilutions bactericidal at 20 and 22°.					
Exposure in minutes ...		15	30	60	120	180	240
E. australiana (Braidwood), acidity = 7°	*a*. (20°) 1 :	400	600	600	900	900	1000
	b. (20°) 1 :	500	600	800	1000	1100	1200
	c. (20°) 1 :	400	500	600	1000	1100	1200(race 6)
	d. (22°) 1 :	500	700	900	1000	1400	—
Probable effective dilution	1 :	400	600	800	1000	1120	1200

It is apparent that phellandrene is more efficient than cineol as a germicide, if the only difference between the two oils lies in the cineol and phellandrene. The low acidity of the Braidwood oil shows that it had been recently distilled, as it had been given out to be. It is one of the most efficient of the oils that have been examined, the only stronger oil being the old specimen of *E. cinerea*, rect.

The Phellandrene Oil of E. dives.—In the absence of pure phellandrene, use was made of the oil of *E. dives*, which consists largely of this constituent. There is also in it a quantity of piperitone, the strongly smelling peppermint substance of the Eucalypts. The oil of *E. dives* is not usually rectified, and is extensively used in the flotation of minerals.*

TABLE xi. *B. coli communis.*

		Dilutions bactericidal at 20°, 21°, and 22°.					
Exposure in minutes		15	30	60	120	180	240
E. dives, crude, acidity = 52°	*a.* (20°) 1 :	200	400	500	600	600	—
	b. (20°) 1 :	300	400	600	600	600	700
	c. (21°) 1 :	200	300	600	700	700	800
	d. (22°) 1 :	400	500	700	800	1000†	1000†
Probable effective dilution at 20° 1 :		300	400	500	600	660	700

†=or weaker.

* The use of this oil as a disinfectant for clothing was tested. It is a sticky oil, and about the worst that could be employed for such purposes, on account of its rather difficult removal with soap and water. This consideration, however, did not occur to the writer until later. A suspension of *M. aureus* in serum was absorbed on cotton mending and dried at 37° for an hour. The infected twists were then inserted in dilutions of *E. dives* and withdrawn at stated intervals (the excess dilution being removed on porous gypsum strips) and inserted into broth. The lethal dilutions were as follows :

	5	10	15	30	60	120	180	240 minutes.
1 :	less than 5		20	20	30	60	80	80

The results indicate that while phellandrene, as exemplified by this oil, is certainly more germicidal than cineol, it is less bactericidal than the phellandrene oil of *E. australiana*, Braidwood. The bactericidal dilutions are somewhat akin to those of the normal variety of *E. australiana*, so that there is something in the oil of the Braidwood variety more potent than phellandrene, possibly the oxidation products of the unknown alcohol.

The Oil of E. Smithii.—This oil contains over 70% of cineol. The other constituents are pinene, with small quantities of a phenol, volatile aldehydes, eudesmol, esters containing butyl-butyrate and a sesquiterpene. It contains neither phellandrene, piperitone, nor aromadendral.

TABLE xii. *B. coli communis.*

		Dilutions bactericidal at 20° and 22°.						
Exposure in minutes ..		15	30	60	120	180	240	300
E. Smithii, rect. Acidity=37°	*a.* (20°) 1 :	300	400	400	500	800	900	—
	b. (20°) 1 :	300	400	500	700	700	900	—
	c. (20°) 1 :	300	300	600	700	800	1000	1000
	d. (22°) 1 :	400	500	700	900	1000	—	—
Probable effect. dilution at 20°, 1 :		300	400	550	700	800	900	1000
E. Smithii, crude. Acidity=35°	*a.* (20°) 1 :	300	300	400	600	600	600	—
	b. (20°) 1 :	200	300	450	600	600	700	800
Probable effective dilution 1 :		200	300	450	600	660	700	—

This is another case in which we have the rectified oil having a stronger disinfecting action than the crude. It is not quite so pronounced as with *E. cinerea*, but there is less room for the formation of substances of an aldehydic nature; the saponifica-

tion number varies from 2·4 to 3·0 as against 14·4 to 24 in the case of *E. cinerea*.

The Oil of E. polybractea.—*E. polybractea*, or "Blue Mallee," is one of the chief sources of the medicinal oil at the present time. The crude oil contains over 70% of cineol, besides pinene and aromadendral. The rectified and crude oils were tested, while a residual oil was included in order to see in how far the presence of aromadendral would influence the germicidal activity.

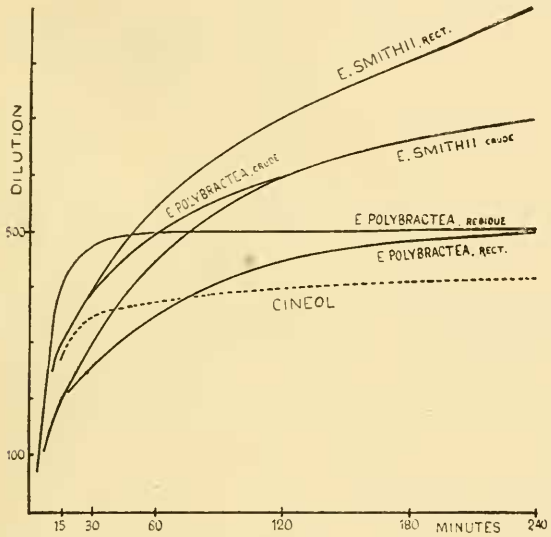
TABLE xiii. *B. coli communis.*

		Dilutions bactericidal at 20°.					
Exposure in minutes ...		15	30	60	120	180	240
E. polybractea, rect. Acidity = 10°	*a.* 1 :	200	200	200	500	500	500
	b. 1 :	200	200	300	300	400	400
	c. 1 :	200	200	300	400	500	500
	*d.** 1 :	200	300	300	400	400	500
Probable effective dilution	1 :	200	260	350	450	480	500
E. polybractea, crude. Acidity = 4°	*a.* 1 :	400	400	500	600	800	800
	b. 1 :	300	400	500	600	600	700
	c. 1 :	300	300	400	500	500	600
Probable effective dilution	1 :	300	400	500	600	650	700
E. polybractea, residue	*a.* 1 :	400	600	600	—	—	—
	b. 1 :	500	500	500	500	500	600
	c. 1 :	400	400	500	500	500	500
Probable effective dilution	1 :	400	480	500	500	500	500

* This test was made after the paper was written with the idea of testing the validity of the earlier tests.

This is an instance where the crude oil is more germicidal than the rectified. The residual oil, the dilutions of which had

the odour of aromadendral, has a curve suggestive of a mixture of aromadendral with an almost inert oil such as sesquiterpene.



Text-fig. 6.

Some Constituents of the Oils.

Cuthbert Hall showed that of all the undiluted constituents of the oils, aromadendral was the most active germicide. In view of this, it seemed advisable to test the aldehyde in aqueous dilutions. Aromadendral has a high boiling point (210°-215°), and largely remains behind when the crude oils of the "Mallees" —*E. polybractea* for example and of the "Box" group of Eucalypts are rectified by steam distillation. At the present time there is no market for these residuals containing the aromadendral, and they are usually thrown away. However, Mr. H. G. Smith was able to obtain a small quantity of the residues, and prepared for me about five c.c. of the aldehyde, which was sufficient to test its properties.

Piperitone, the ketone of the "Peppermint" group of Eucalypts, was tested about the same time, and I included the oils of *E. nova-anglica*, the chief constituent of which is a sesquiterpene,

of *E. dextropinea*, and of *E. lævopinea*, both of which consist very largely of pinene.

TABLE XIV. *B. coli communis.*

	Dilutions bactericidal at 20, 21, and 22°.					
Exposure in minutes ...	15	30	60	120	180	240
Aromadendral *a.* (20°) 1 :	1600	—	2400	2800	2800	2800
b. (20°) 1 :	1800	2200	2600	2600	3000	--
c. (21°) 1 :	1600	2600	2800	2800	3000	3000
d. (22°) 1 :	—	2800	2800	2800	2800	—
Prob'le effect. dilution at 20° 1 :	1700	2200	2600	2800	2900	3000
Aromadendral and cineol, equal parts (20°) 1 :	800	—	1400	1400	—	1400
Piperitone (20°) 1 :	400	--	450	500	500	500
E. nova-anglica. Acidity = 33° (20°) 1 :	40	—	60	—	60	—
E. dextropinea. Acidity = 68·5° (20°) 1 :	40*	80	100	120	130	140
E. lævopinea. Acidity = 94° (20°) 1 :	40	100	140	200	200	200

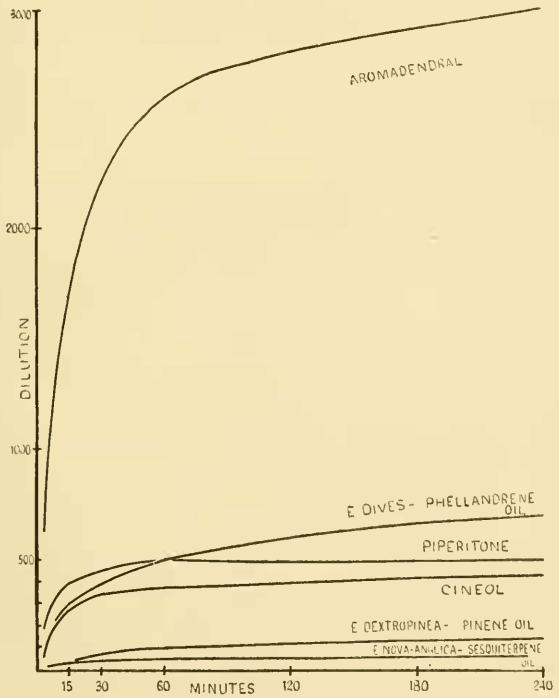
* = or stronger.

There can be no doubt that of all the oils and their constituents that have so far been tested in aqueous dilutions, aromadendral is by far the most efficient germicide. It is about seven times stronger than cineol. Its bactericidal power does not appear to be reduced to any great extent beyond the expected proportion when it is mixed with cineol, so that its presence in any oil should raise the efficiency of that oil.

Previous experiments with the sesquiterpene oil of *E. nova-anglica* showed that, when dissolved in olive oil, it was the most inert towards *B. coli communis*. This was borne out by its behaviour in aqueous dilutions; its germicidal power is very feeble.

Piperitone is stronger than cineol, but not to any great extent.

Pinene, as represented by the oils of *E. dextropinea* and *E. lævopinea*, has a comparatively feeble action, although slightly better than sesquiterpene.



Text-fig. 7. The Constituents of the Eucalyptus Oils.

The Oils of E. cneorifolia and E. citriodora.—The oil of *E. cneorifolia*, one of the "Mallee" group from Kangaroo Island, contains cineol and aromadendral with a small amount of sesquiterpene. It should give high dilution numbers, and the expectation was fulfilled. The oil of *E. citriodora* contains 91% of the aldehyde citronellal and a small amount of pinene, but no cineol.

TABLE XV. *B. coli communis.*

	Dilutions bactericidal at 20°.						
Exposure in minutes...	15	30	60	120	180	240	300
E. cneorifolia, crude.							
Acidity = 1° *a.* 1 :	300	500	800	900	1000*	1000*	—
b. 1 :	300	500	800	800	1100	1100	1100
Probable effective dilution 1 :	300	500	800	1000	1080	1100	1100
E. citriodora, crude.							
Acidity = 140° *a.* 1 :	300	300	600	1000	1000	1200	1200
b. 1 :	400	400	600	800	1100	1200	—
c. 1 :	400	400	800	1000	1200	1200	1200
Probable effective dilution 1 :	330	500	700	900	1020	1140	1200

* = or weaker.

Both these oils are good disinfectants; they are the best of the crude oils that have been examined; the reason for their excellence is to be found in the presence of the aldehydes, aromadendral and citronellal. Indeed, from the work that has been done, it is clear that a comparatively high germicidal activity can only be expected in those oils containing substances of an aldehydic nature. The pinenes and sesquiterpene are, inferentially, poor disinfectants; cineol is moderate, phellandrene and the ketone piperitone are better, and the aldehydes are the best.

SUBSIDIARY NOTES.

a. The races in the stock culture.—During the research, the stock culture of *B. coli communis* was plated to see if it had retained its purity. The plates showed a pure culture. One of the colonies was picked out and used. As it appeared to be much more sensitive to the action of the Eucalyptus oils than the stock culture, a direct test was made, using the 2nd hour oil of *E. australiana*.

TABLE xvi. Races of *B. coli communis*.

Exposure in minutes ...	15	30	60	120	180	240
Stock culture (mixed races) 1 :	400	400	400	400	600	600
New culture (race 1) 1 :	600	900	900	1000	1000	1200

The new culture was almost twice as sensitive to the action of the disinfectant as the stock culture with which the work had been done.

The result of the test led to the trying of nine colonies, picked out at random from a plate containing about fifty colonies, and, in order to economise labour and material, the time test with single dilutions was employed. A spiral loop of broth culture (7 milligrams) was put into 2 c.c. of diluted disinfectant and portions were withdrawn at intervals of 5, 10, 15, and 30 minutes. Longer exposures were all negative.

TABLE xvii. Minutes required to destroy *B. coli communis*.

	E. australiana, 2nd hour. 1 : 600.	*E. australiana*, rect. 1 : 400.
Stock culture	15	15
Race 1	10	10
Race 2	15	30
Race 3	30	10
Race 4	15	10
Races 5, 8, and 9	15	15
Race 6	30	30
Race 7	30	15

Race 1, the behaviour of which had led to the test, proved to be the most sensitive of all the races. Race 7 which, from its resistance to the 2nd hour oil of *E. australiana*, promised to be hardy, did not justify the promise either in the second part of the test or in Table ix., where test *c*. was made with the stock culture and test *d*. with race 7 upon the same day and at the same time. Race 6 appeared to be the most resistant of all the races, and it was tried with *E. australiana* (Braidwood) upon the same day and at the same time. The result showed that it

had the same resistance as the stock culture. In Table x., test *b.* was made with the stock culture and test *c.* with race 6.

The stock culture clearly contained a mixture of races, the most resistant of which have played their part in the recorded experiments. The germicidal dilutions are those which destroy all the bacteria in certain times, and naturally the most resistant bacteria will be the last to succumb.

b. The Effect of Mass Infection —The lethal dilutions for the stock culture in the previous test with the races of *B. coli communis* are not in agreement with the results tabulated elsewhere. This is explained by the fact that the infected broth added to the 2 c.c. of disinfectant weighed 7 milligrams, while elsewhere the infecting material weighed 25 milligrams (the droplet from a standard pipette). The proof of this is found in the following:

TABLE XVIII. *B. coli communis.*

Exposure in minutes ...		15	30	60	120	180	240
E. australiana, 2nd hour.							
Infection, 25 mgrms.	1 :	400	400	500	600	600	800
7 mgrms.	1 :	600	600	700	700	1000	1000

It confirms the well known rule of mass infection, *i.e.*, the greater the number of bacteria, the stronger must be the disinfectant to destroy them. It emphasises the importance of keeping to one method of technique in doing a set of experiments.

c. The Effect of Acidity.—In a preliminary test, it was found that when the oil of *E. cinerea* was diluted with water containing 1° of alkalinity as sodium bicarbonate, it was less germicidal than when neutral distilled water was employed. It is possible that the converse of this holds, and that slightly acid water would increase the germicidal power. There is also the possibility that an acid oil may be more toxic on account of the acidity conferred upon the diluting water by the acid of the oil. The elucidation of these two points was attempted.

The chief and probably the most active free acid in the oils is acetic, and accordingly this acid was tested to see if it had any bactericidal power of its own. A solution was prepared by

taking 1 c.c. of glacial acetic acid and diluting it to 50 c.c. This was found to be equivalent to $\frac{N}{2\cdot77}$. It was diluted progressively from 5 to 500 times, and tested with *B. coli communis* in the usual manner. All the tests were positive with exposures from one to four hours. Acetic acid is, therefore, inactive in dilutions ranging from zero up to $\frac{N}{13\cdot8}$, that is, up to 72° of acidity. It follows that any assistance given by the acid in the dilutions of the oils cannot be due to the acid directly, but to a condition that the acid brings about.

The next experiment was made with cineol which was acidified with acetic acid so that it contained 92·5° of acidity. This is close to the acidity of the specimen of the rectified oil of *E. cinerea* which had been previously tested (Table iv.).

The acidified oil was a little more toxic, but the differences were comparatively slight, due possibly to the fact that, when the oil is diluted 300 or 350 times, the original acidity is reduced to 0·26° (= a quarter of a c.c. of normal acid per litre) which is a comparatively slight acidity.

The effect of using acidified water in place of neutral distilled water was then investigated. A supply of recently distilled oil of *E. polybractea* with a natural acidity of 10° was used. Dilutions were made with water acidified with acetic acid and containing varying degrees of acidity.

TABLE XIX. *B. coli communis.*

	E. polybractea. rect. Dilutions bactericidal at 20°.				
Acidity of water.	30 min.	60 min.	120 min.	180 min.	240 min.
b. neutral	200	300	400	600	600
1°	300	500	700	1000	1000
a. 1°	400	500	800*	800*	800*
5°	400	500	600	800	1400
15°	500	600	800	800	1800

* = or weaker.

The results are somewhat irregular, but as a whole they show that an acid condition increases the potency of the oil in aqueous

dilutions. For this reason, it would be a mistake to employ ordinary tap-water when making dilutions, as its slight alkalinity would tend to reduce the germicidal efficiency.

Since phenol behaves as a weak acid, it is likely that its activity will be enhanced in the presence of dilute acid. A preliminary test showed that this was so, for with 10° of acidity, as acetic acid, dilutions up to 1 : 200 were germicidal in 30 minutes. A test made at the same time with 11° (corrected) of alkalinity as sodium bicarbonate gave normal numbers up to an hour, and at four hours the lethal dilution was 1 : 180, as against 1 : 140 with neutral water. Alkali itself has no action when used in small amounts, for a control test showed that water with 22° of alkalinity as sodium bicarbonate was inactive in four hours.

Thus the addition of alkali or of acid to the water used for dissolving the phenol, increases the efficiency, and of these, the acid is the more powerful.

The effect of acidity upon the action of phenol was confirmed in the following experiment. Solutions of acetic acid were prepared to give 1° and 10° of acidity, but when the pipette was checked it was found that the number of drops per gram differed with the strength of the acid. Solutions of $\frac{N}{7\cdot5}$ strength gave 72 drops per gram, while those of $\frac{N}{0\cdot75}$ gave 90 drops. The actual acidity was therefore weaker.

TABLE XX. *B. coli communis.*

	Phenol, acidified dilutions bactericidal at 20°.			
Exposure in minutes ...	15	60	180	300
Acidity of water				
a. 0·96° 1 :	120	160	220	260
7·26° 1 :	140	220	300	340*
b. 10° 1 :	140	220	340	380
neutral as previously determined 1 :	90	120	145	150

* = or weaker.

There is nothing new in this example of the influence of acidity in increasing the activity of phenol, for Delepine* quotes an experiment showing that the presence of acetic acid in a solution of phenol in the ratio of 1 : 400 (= 41° of acidity) increased the activity of the disinfectant by 140/70 in five minutes at 17°. Hydrochloric acid in the ratio of 1 : 8000 (= 3·4°) increased it by 80/70 in the same time. Hailer† says that acids increase the disinfecting power of phenols in the order oxalic, sulphuric, acetic, tartaric, citric, boric; the last has scarcely any action. As having a bearing upon the matter, it is known that a faint acidity causes a medium to be easily sterilised by heat. Currie, for example, added 4 c.c. of seminormal hydrochloric acid per litre to obtain the sterilisation of a saccharose medium in one steaming.

d. Water increases the efficiency of the oils.—The value of water in enhancing the germicidal effect of cineol and reasonably of Eucalyptus oils, is shown by the fact that *B. coli communis*, when suspended in serum, was destroyed by a 50% dilution of cineol in olive oil where the only water present was contained in the serum. When an aqueous dilution of cineol was given the same time to act, the bacteria were destroyed in a dilution of 1 : 415, *i.e.*, 50%, with a trace of water and 0·24% with much water.

An experiment was designed to see in how far bacteria impregnated on cotton could survive the vapour of cineol at ordinary temperatures (23° to 26°C.), the idea being to determine if cotton masks such as were worn during the pneumonic influenza epidemic would be sterilised when enclosed in a vessel containing cineol vapour. Some strands of cotton were infected with seven milligrams of *coli*-infected broth, and immediately suspended in cineol vapour (see p.91); others were dried in the air for an hour before being suspended in the same manner. The moist strands were found to be sterile in 60 minutes, while the dry strands were sterile in three but not in two days. Obviously, if

* Journ. Soc. Chem. Ind., 1910, 1344.
†Abstr. Journ. Soc. Chem. Ind., 1910, 514.

the masks were slightly damped, they could be depended upon as being sterile after a night's exposure to the vapour.

Conclusions.

The germicidal value of the Eucalyptus oils varies with the kind of oil and also with the particular specimen. While Baker and Smith have shown that the major chemical constituents are wonderfully uniform, so much so that from a consideration of the oil they are able to recognise the affinities of species of the Eucalypts, the minor constituents may vary considerably, and these seem to have a pronounced influence upon the germicidal power. To realise this, one has only to consider the variations in the bactericidal power of the oil of *E. cinerea*. The rectified oils were germicidal in an hour with dilutions of 1 : 900, 1 : 500, and 1 : 340 according to the individual specimen. These were not all of the same age. The most potent was the oldest, and it was the most acid. From this we infer that the degree of acidity is to a certain extent an index of the age and, to some extent, an index of the disinfecting power. At any rate, with an oil having a high saponification number such as that of *E. cinerea*, it is to be expected that, as time goes on, the oil becomes oxidised and the constituents decomposed, yielding oxidation products such as aldehydes and acids. The aldehydes seem to possess high germicidal values, one of them, aromadendral for example, is a very powerful disinfectant.

But the acidity is only a rough index, for the three specimens had their acidities in the ratio, 95 : 18 : 26, from which we see that, when the acidity is low, there is no indication given as to the disinfecting action. Possibly the original acidity of the oil should be taken into account.

It is customary in dealing with the germicidal activities of disinfectants to refer to them in terms of a standard disinfectant such as phenol, and accordingly the phenol-coefficients of these oils that have been tested, were calculated and appear in the following Table in which the rectified oils have been grouped in the approximate order of their efficiency. The same applies to the crude oils and to the oil constituents.

TABLE XXI. *B. coli communis.*

Acidity in degrees.	Iodine number in seconds.	Exposure in minutes	Phenol-coefficients at 20°. 15	30	60	120	180	240
95	5	*E. cinerea*, rect.	4·4	5·8	7·6	10·0	11·7	13·2
18	25	,, ,, ,, ...	2·2	3·2	4·2	5·4	6·2	6·6
26	90	,, ,, ,, ...	1·7	2·4	2·9	3·2	3·1	—
37	14	*E. Smithii*, rect.	3·3	3·9	5·0	5·2	5·5	5·9
10	000*	*E. polybractea*, rect.	2·2	2·5	3·0	3.3	3·3	3·3
7	12	*E. australiana* (Braidwood), crude ...	4·4	5·8	6·8	7·4	7·7	7·9
41	75	*E. cneorifolia*, crude	3·3	4·8	6·8	7·4	7·4	7·2
140	000	*E. citriodora*	3·7	4·8	5·9	6·7	7·0	7·5
5	15	*E. australiana*, 1st hour	2·5	3·4	3·9	3·9	3·8	3·6
10	11	,, ,, ,, ,, ...	2·8	3·1	3·4	3·6	3·6	3·6
150	12	,, ,, 2nd hour	3·3	3·9	4·2	4·2	4·1	3·9
52	34	*E. dives*, crude	3·3	3·9	4·2	4·4	4·5	4·6
4	30	*E. polybractea*, crude	3·3	3·9	4·2	4·4	4·5	4·6
35	32	*E. Smithii*, crude	2·2	2·9	3·9	4·4	4·5	4·6
86	30	*E. cinerea*, crude	2·5	2·9	3·1	3·2	3·3	3·2
12	150	,, ,, ,, ...	1·7	1·9	2·3	2·6	2·7	2·8
81	000	,, ,, ,, ...	1·7	1·7	2·1	2·6	2·7	2·6
—	000	Aromadendral	18·9	21·1	21·1	20·7	20·0	19·7
—	55	Piperitone	4·4	4·1	3·9	3·7	3·4	3·3
2	000	Cineol†	3·1	3·4	3·3	3·1	2·9	2·8
52	34	Phellandrene oil (*E. dives*)	3·3	3·9	4·2	4·4	4·5	4·6
68	000	Pinene oil (*E. dextropinea*)...	0·4	0·8	0·9	0·9	0·9	0·9
33	75	Sesquiterpene oil (*E. nora-anglica*) ...	0·4	0·5	0·5	0·4	0·4	0·4
		Phenol...	1·0	1·0	1·0	1·0	1·0	1·0

*000 = over 180 seconds. † Cineol at 2½ minutes = 0·07, at 5 minutes = 1·0, at 7½ minutes = 2·6 and at 10 minutes = 3·0.

Certain text-books give the coefficient of the oil of *E. globulus* as about 4. Martindale* emulsified the oils of *E. globulus* and of *E. amygdalina* in a solution of soap, and, taking the average of the coefficients at two and at thirty minutes, found them to be 3·55 and 4·35 respectively. As soap, however, is itself a disinfectant, it is probable that these numbers are high. Cavel*, working with the bacteria from a septic cyst, determined the maximum doses of essential oils, dissolved in acetone or alcohol, necessary to prohibit growth in infected media. Essence of Eucalyptus was lethal in 2·75 parts per 1,000, and phenol in 5·6 parts. This gives a phenol-coefficient of about 2.

According to Baker and Smith, *E. globulus* belongs to the same group of Eucalypts as *E. cinerea* and *E. Smithii*, the rectified oils of which have high coefficients. It is curious that, while the rectified oils of these trees are among the most efficient, the crude oils are among the least, judging by the coefficients at the end of the first hour. Even at the end of 30 minutes, which is, I think, a fair period for purposes of comparison, the same holds.

We are led to believe that the rectification of an oil, such as those that were tested, gives us a product containing a higher proportion of cineol. It is, therefore, strange to find that one of the samples of *E. cinerea* and one of *E. polybractea* have a lower coefficient than cineol. In the case of *E. polybractea*, rectification appears to have removed the high boiling aromadendral from the lower boiling cineol and pinene; the sample gave the coefficient of a mixture of 50% of cineol and 50% of pinene.

Cuthbert Hall, from his observations upon the behaviour of the oils towards iodide of starch paper, considered that the ozone content of the oils was an index of their germicidal power. I showed that this did not hold for the Eucalyptus oils when they were dissolved in olive oil. With regard to the iodide of starch reaction and the activity of the oils when emulsified in water, there does appear to be some relation when individual oils are considered. The three specimens of rectified oil of *E. cinerea*, and

* Abstr. Journ. Soc. Chem. Ind., 1910, 1470.

† Compt. Rend., (20), 21/5/18, p.827.

the three crude oils of the same species have their phenol-coefficients inversely as their iodide of starch times. The acidities do not show any such order. But when we take the oils as a whole, we find that there is no relation, indeed in view of the high germicidal power of aromadendral, we are driven to the conclusion that the chemical constituents of the oils, exclusive of any oxidising body, are the sources of the germicidal substances, and determine the disinfecting efficiency. The hydrolysis of the esters and the oxidation of the resulting alcohols is undoubtedly the reason for the enhanced efficiency of the older oils.

As disinfectants, the rectified oils do not appear very promising. The older oils of *E. cinerea* are certainly good, but those recently distilled are not. The rectified oil of *E. polybractea* is comparatively poor, while *E. Smithii* is good.

With regard to the crude oils, the question of the price comes in. Mr. H. G. Smith informed me that at the end of the year 1918, the oils of *E. cneorifolia, E. polybractea,* and *E. cinerea* cost from 1s. to 1s. 3d. per lb. at the still. The oil of *E. australiana,* 1st hour, cost 1s. to 1s. 2d., and of *E. dives* and *E. australiana* (Braidwood), 7d. to 8d. Rectification would add about 2d. per lb. to these figures. Thus the two most effective crude oils, *E. cneorifolia* and *E. australiana* (Braidwood) cost about 1s. 3d. and 7½d. each per lb. respectively. *E. australiana* (Braidwood) is thus the cheaper, and, at the same time, the better disinfectant. It is quite a pleasant oil to work with, and, although containing phellandrene, it has not the sticky character of the oil of *E. dives.*

On account of the difficulty of maintaining an emulsion with water, these oils will probably not displace the tar-products, which are more easily emulsified when in strong dilutions. But there are situations in which the use of the Eucalyptus oils would be preferable. The disinfection of the walls of a room, for example, by spraying, is a case in which a Eucalyptus oil, such as *E. australiana* (Braidwood) could be recommended. The oil has a phenol-coefficient of 5·8, and a dilution of 1:600, destroys *B. coli communis* in half-an hour. An emulsion of, say, 1:400 does not

separate readily, and it is more efficient than a 1:80 solution of phenol. As it is at least five times more effective than phenol, and very much cheaper, it should find considerable use in spraying rooms and in disinfecting clothing or such materials as are free from oils or fatty substances, in which the Eucalyptus oil is more soluble than in water. The necessity for the use of water in conjunction with the undiluted oils has already been emphasised.

Summary.—The Eucalyptus oils are irregular in their action upon *B. coli communis*, and duplicate experiments may show a considerable amount of variation.

Cineol begins to act in about a minute and a half; phenol acts instantly.

The curves of cineol and phenol cross in five minutes with a dilution of 1:75 at 20°.

The phenol coefficient of cineol in 15 minutes at 20° is 3·1; it rises to 3·4 in 30 minutes, and then slowly declines to 2·8 in 4 hours.

Aromadendral is the most active of the constituents of the oils. The phenol-coefficient is 21·1 in 30 minutes.

The next most active is piperitone (4·1), and possibly phellandrene.

Pinene and sesquiterpene are low (0·8 to 0·5).

The rectified oils of *E. cinèrea* and *E. Smithii* are more efficient than the crude oils.

In the case of the oil of *E. cinerea,* this appears to be due to the hydrolysis of the esters and the subsequent oxidation of the alcohols to aldehydes.

Treatment with alkali did not reduce the efficiency of the acid, rectified oil.

The addition of acetic acid to the crude oil doubled the germicidal power in the course of $3\frac{1}{2}$ months.

The germicidal activity of the rectified and crude oils of *E. cinerea* is proportional to the starch-iodide reaction, and not to the acidity, but this does not hold for the oils as a class.

The rectified oil of *E. polybractea* is less efficient than the crude oil.

This may be due to the elimination of aromadendral during rectification.

The oil of the Braidwood variety of *E. australiana* is the best and cheapest disinfecting oil (phenol-coefficient = 5·8 in 30 minutes.)

The oil of *E. cneorifolia* was the second best crude oil tested (phenol-coefficient = 4·8 in 30 minutes); its activity is probably due to its aromadendral content.

As in the case of phenol, the addition of acid to the water used in emulsifying the oils greatly increases the germicidal activity.

I have to acknowledge the kindness of Messrs. Baker and Smith, of the Technological Museum, in supplying me with specimens of the oils, of Mr. H. G. Smith in giving details connected with the oils, and of Mr. W. W. L'Estrange in giving much valuable assistance.